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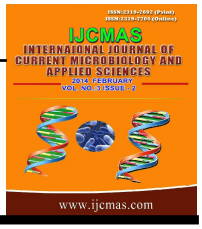

Original Research Article

Bioactivity of stigmasterol isolated from the aerial part of *Spillanthes acmella* (Murr) on selected microorganism

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A B S T R A C T

The Stigmasterol was isolated from Spillanthes acmella Murr previously, which was subjected to antimicrobial activity test using the following clinical microbes to determine its biological activities; Methycilin Resistant *Staphylococcus aureus (MRSA), Staphylococcus aureus, Streptococcus pyrogenes, Bacillus subtilis, Corynebacterium ulcerans, Escherichia coli, proteus mirabilis, proteus vulgaris, Pseudomonas aureginosa, Salmonella typhi, Shigellia dysenteriae, Candida albicans, Candida virusei*, and *Candida tropicalis*. The zone of inhibition was observed to be between 20 to 24 mm with Bacillus Subtilis having the largest zone of inhibition of 24 ml.The Minimum Inhibitory Concentration (MIC) and The Minimum Fungicidal Concentration (MFC) was determined. It was found that the MFC was higher than that of standard drug Fluconazole. This compound has a wide range of broad spectrum over the standard drug against the entire test organism.

Keywords

Bioactivity; Stigmasterol; Spillanthes; antimicrobial; inhibition; MIC.

Introduction

Spillanthes acmella Murr is a flowering herb in the family Asteraceae. It is known as toothache plant or pancress as the leaves and the flower heads contain analgesic agent Spilanthol that is used to numb toothache. The fresh and cooked leaves are combined with chilies and garlic to add flavour and vitamins to foods (Miyazawa *et al*; 2006). Some chemical constituents have been isolated from the plant such as Spilanthol an alkyl amide (Gokhale and Bhide, 1945). This is responsible for the saliva inducing effect of the plant, and some triterpenoids (Ramsewak *et al*; 1999). The leaves along with alum are recommended as emetic (Gill, 1992). Because of this observation and the regular uses of the plant by the natives prompted this research possibly to find out if there could be more metabolites that can be of pharmacological relevance.

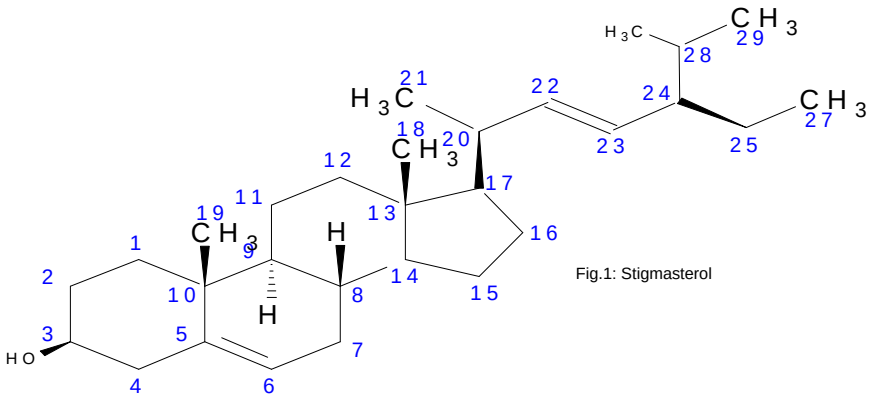

Fig.1: Stigmasterol

Table.1 Zone of inhibitions (mm) for Stigmasterol on the test microorganism

Test Organism	**SPEC-1**
MR*SA*	0
Staphylococcus aureus	21
Streptococcus pyrogenes	22
Bacillus subtilis	24
Corynebacterium ulcerans	0
Escherichia coli	21
Proteus mirabilis	20
Proteus vulgaris	21
Pseudomonas aureginosa	0
Salmonella typhi	21
Shigellia dysenteriae	21
Candida albicans	21
Candida virusei	20
Candida tropicalis	20

Table.2 Minimum Inhibitory Concentrations (**µg/ml**) of Stigmasterol on the test microbes

Test Organism	**50**	**25.5**	**12.5**	**6.25**	**3.125**
MR*SA*					
Staphylococcus aureus	_	_	O*	+	++
Streptococcus pyrogenes	_	_	O*	+	++
Bacillus subtilis	_	_	O*	+	++
Corynebacterium ulcerans					
Escherichia coli	_	_	O*	+	++
Proteus mirabilis	_	_	O*	+	++
Proteus vulgaris	_	_	O*	+	++
Pseudomonas aureginosa					
Salmonella typhi	_	_	O*	+	++
Shigellia dysenteric	_	_	O*	+	++
Candida albicans	_	_	O*	+	++
Candida virusei	_	_	O*	+	++
Candida tropicalis	_	_	O*	+	++

Key: **-** =No colony growth, **O*** =MIC, **+** =light growth, **++** = Moderate colonies growth

Table.3 Minimum Bactericidal/Fungicidal Concentrations (µg/ml) of SPEC-1 on the test microbes

Test Organism	**50**	**25.5**	**12.5**	**6.25**	**3.125**
MRSA					
Staphylococcus aureus	O*	+	++	+++	++++
Streptococcus pyrogenes	O*	+	++	+++	++++
Bacillus subtilis	O*	+	++	+++	++++
Corynebacterium ulcerans					
Escherichia coli	O*	+	++	+++	++++
Proteus Mirabilis	O*	+	++	+++	++++
Proteus Vulgaris	O*	+	++	+++	++++
Pseudomonas aureginosa					
Salmonella typhi	O*	+	++	+++	++++
Shigellia dysenteriae	O*	+	++	+++	++++
Candida albicans	O*	+	++	+++	++++
Candida virusei	O*	+	++	+++	++++
Candida tropicalis	O*	+	++	+++	++++

Key: **-** =No colony growth, **O*** =MBC/MFC, **+** =Scanty colonies growth, **++** = Moderate colonies growth, **+++**= Heavy colonies growth

Table.4 Minimum Inhibitory Concentrations (µg/ml) of Fluconazole on the test microbes

Test Organism	**50**	**25.5**	**12.5**	**6.25**	**3.125**
MRSA	□	□	□	□	□
Staphylococcus aureus	□	□	□	□	□
Streptococcus pyrogenes	□	□	□	□	□
Bacillus subtilis	□	□	□	□	□
Corynebacterium ulcerans	□	□	□	□	□
Escherichia coli	□	□	□	□	□
Proteus mirabilis	□	□	□	□	□
Proteus vulgaris	□	□	□	□	□
Pseudomonas aureginosa	□	□	□	□	□
Salmonella typhi	□	□	□	□	□
Shigellia dysenteriae	□	□	□	□	□
Candida albicans	_	_	O*	+	++
Candida virusei	_	_	O*	+	++
Candida tropicalis	_	_	O*	+	++

Key: **-** =No colony growth, **O*** =MIC, **+** =light growth, **++** = Moderate colonies growth, □=Resistant.

Materials and Methods

Isolation and characterization

Stigmasterol (Fig.1) was isolated from the aerial parts of *Spillanthes acmella* as a white solid crystal by vacuum liquid chromatography (VLC).This was characterized and established by 1D and 2D NMR spectroscopic analysis and by direct comparison of the data obtained with those reported in literature to be Stigmasterol (Isah *et al*; 2012).

Anti-microbial activity of the Stigmasterol on selected microbes

The antimicrobial screening of the compound was carried out on the following clinical selected microbes; Methicillin Resistant *Staphylococcus aureus(*MR*SA), Staphylococcus aureus, Streptococcus pyrogenes, Bacillus subtilis, Corynebacterium ulcerans, Escherichia coli, Proteus mirabilis, Proteus vulgaris, Pseudomonas aureginosa, Salmonella typhi, Shigellia dysenteric ,Candida albicans, Candida virusei, and Candida tropicalis* to determine their activities based on the traditional uses of this plant. The cork and bore diffusion method (Bauer *et al*; 1966), (Barry and Thornsberry, 1985) was used in the anti-microbial screening

Results and Discussion

The zone of inhibition was observed to be between 20 to 24 mm (Table 1) .This shows that *Bacillus subtilis* is the most sensitive organism with the largest zone of inhibition of 24 mm while the smallest zone of inhibition was observed for *proteus mirabilis, Candida virusei* and *Candida tropicalis* at 20 mm. The MIC and MBC/MFC in Table 2 and 3 showed that all the sensitive organism has MIC values at 12.5µg/ml, while the MBC/MFC were observed for the microbes at concentration higher than that of the MIC at 50 µg/ml. The MFC was equally observed at a concentration higher than that of the standard drug Fluconazole (Table 4).It was equally observed that SPEC-1 has wide range of broad spectrum over the standard drug against the organisms tested. It was generally noted that SPEC-1 can be use as an antifungal agent in the treatment of fungal related diseases.

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